THE EFFECT OF CLA SUPPLEMENTATION ON FAT DEPOSITION AND LEAN MUSCLE MASS IN HORSES

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DEDICATION

For the One who has given me the passion for research and the strength to persevere through the hardships to achieve my goals. This would not have been possible without Him and to Him be the glory.

ABSTRACT

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Fatty acids are utilized within the equine industry to increase the caloric density of a diet as well as replace soluble carbohydrates. Omega-3 fatty acids are the most commonly supplemented fatty acids due to their potential health benefits; however, palatability limitations have spurred an investigation into alternative fatty acids like conjugated linoleic acid (CLA). CLA has shown health benefits similar to that of omega-3s, but the effect of CLA on equine fat deposition and lean muscle mass has yet to be established. The purpose of this investigation was to evaluate the effects of supplementing elevated levels of CLA on both lean muscle mass and fat deposition in young growing horses. In phase I of this study, 10 Quarter horses were fed between 5.0% and 10.0% of the concentrate diet, increasing the supplementation level every 3 d to determine the maximum inclusion rate of CLA in horses. In phase II, 9 Quarter horses were separated into 2 treatment groups fed either a control diet of soybean oil or CLA (n = 4 and 5/group, respectively) for 12-wks with BW and sex evenly distributed across treatments. Diets were formulated to be isocaloric and isonitrogenous and each treatment was offered at 0.015% BW/d. Growth measurements were collected weekly; rump fat thickness (RFT), ribeye area (REA), back fat (BF) and intramuscular fat (IMF) were measured on d 7, d 42, and d 84 of the feeding period. The MIXED procedure in SAS was used with repeated measures to detect differences in growth performance and ultrasound measurements. There were no differences in performance characteristics or fat content between treatment groups ($P > 0.05$). In order to account for initial differences

between treatments ($P < 0.05$); REA between the 17th and 18th ribs (REA17) was run with d 7 as a covariate. Mean REA17 tended to be higher in CLA supplemented horses ($P < 0.07$) when compared to controls. These results suggest that in an equine model, CLA does not affect growth performance or fat deposition, but may increase lean muscle mass in young growing horses. Further studies examining these effects over longer supplementation periods or in obese or insulin-resistant horses may offer insight to potential benefits of CLA in the horse.

KEY WORDS: Conjugated linoleic acid, CLA, Horses, Fat deposition, Lean muscle mass

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PREFACE

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CHAPTER I

Introduction

Horses are considered hindgut fermenters and have the ability to digest cellulose and hemicellulose that other classic monogastrics, like humans and swine, cannot due to specific microbes acting within a well-developed cecum. It is common practice within the performance horse industry to feed soluble carbohydrates in the form of concentrates to meet digestible energy requirements. However, even with adequate access to forages, these high starch diets can cause starch overload, a condition that occurs when soluble carbohydrates are not digested in the small intestine and as a result travel undigested through the small intestine before moving into the cecum, where it undergoes fermentation. The fermentation of starch causes a sharp drop in cecal pH, which can kill the microbes responsible for fiber digestion and may result in colic, laminitis, or founder. Lipid supplementation is a safer alternative to high starch diets due to its high energy density, allowing for increased caloric intake while preventing the starch overload that could result from feeding a high concentrate diet alone. The equine industry utilizes high-fat diets in several ways, such as in pregnant or lactating mares, performance horses, and horses with a high sensitivity to starch or glucose.

Similar to what is seen with human nutrition, some fats are healthier to supplement in horses than others. For instance, omega-3 fatty acids are known to elicit a number of health benefits and as such are the preferred ingredients of commercial fat supplements over their omega-6 counterparts, even though both are essential nutrients and must be included in the diet daily. There are a number of different fat supplement products on the market that are derived from a variety of sources with varying fatty acid

composition. Omega-3 fatty acids are typically supplied in the diet from marine-based sources such as menhaden fish oil or vegetable derived flaxseed oils. However, these common sources of omega-3s have poor conversion efficiency into active ingredients as well as palatability limitations. One fatty acid that has garnered significant scientific attention in the past few decades for its potential health benefits and improved palatability is conjugated linoleic acid (CLA). It is found naturally in meat and dairy products and is marketed as a health supplement in both humans and livestock.

Previous study regarding CLA supplementation in other species, like swine and rats, has shown a number of health benefits, such as the reduction body fat accumulation. However, its use in horses has been minimal to this point and as such; its effects in an equine model are not fully known. The objective of the current study is to determine the maximum inclusion rate of supplemental CLA to young developing horses and to evaluate the effects of supplementing elevated levels of CLA on body composition. Demonstrating the positive physiological benefits of a CLA-supplemented diet will have significance to the health and well-being of all horses, regardless of breed or discipline. In particular, the ability of CLA to reduce the accumulation of adipose tissue could have a significant impact on horses with equine metabolic syndrome (EMS), a disease associated with insulin resistance, obesity, and subsequent laminitis. Additionally, CLA could have applications within various equine disciplines, increasing lean muscle mass and modulating the fat accumulation in young growing horses, competitive performance horses, and working horses.

CHAPTER II

Literature Review

Conjugated linoleic acid (CLA) refers to a group of positional and stereoisomers of conjugated octadecadienoic acid (18:2), or linoleic acid (Headley et al., 2012; Lehnen et al., 2015; Ostrowska 1999; Wang and Lee, 2013). It is naturally found in beef, lamb, and dairy products, predominantly as the c9,t11-CLA isomer, also known as rumenic acid, which is formed as an intermediate in the biohydrogenation of polyunsaturated linoleic acid to saturated stearic acid by the rumen bacteria *Butyrinvibrio fibrisolvens*, *Lactobacillus acidophilus*, *Propionibacterium freudenreichi*, and others (Belury, 2002; Derakhshande-Rishehri et al., 2014; Eftekhari et al., 2013; Kim et al., 2016; Koba and Yanagita, 2014; Lehnen et al., 2015; Thiel-Cooper et al., 2001; Wang and Lee, 2013). While CLA is made up of several isomers of linoleic acid, previous studies have shown that specific isomers are responsible for the different physiological effects attributed to CLA supplementation. Along with the c9, t11-CLA isomer, the t10, c12-CLA is the other important isomer exhibiting positive health effects. For instance, it has been shown that the t10,c12-CLA isomer is primarily responsible for the reduction of body fat gain, whereas both the c9,t11- and t10,c12-CLA isomers appear to be effective in inhibiting carcinogenesis (Belury, 2002; Kim et al., 2016; Koba and Yanagita, 2014; Pariza, 2004; Wang and Lee, 2013). Synthetic preparation of CLA will produce roughly equal amounts of both predominant forms of CLA as well as a small amount of other minor isomers, such as t9,t11-CLA. While these other isomers may also exhibit additional health benefits, c9, t11 and t10, c12-CLA are the main isomers in question. The method of action is dependent on both the specific isomer and what physiological process it affects,

and in some cases, is still unclear (Kim et al., 2016; Lehnen et al., 2015; Wang and Lee, 2013).

Like any other supplemental oil, CLA isomers will accumulate in tissues when fed and are readily metabolized by several pathways. CLA metabolites have been identified in the liver, and mammary tissue of rats and the adipose tissue of humans (Belury, 2002). CLA isomers are metabolized similarly to linoleic acid – producing conjugated arachidonic acid (20:4) through a series of desaturase and elongase reactions. Additionally, CLA can be oxidized to β-oxidation products (16:1 and 16:2), although the role these metabolites play in the modulation of adipose tissue mass, glucose sensitivity, and carcinogenesis has yet to be elucidated (Belury, 2002). Pariza (2004) reviewed previous literature examining the effects of CLA supplementation in various species, with some researchers concerned that CLA could potentially induce insulin resistance, fatty liver, or lipid peroxidation. Despite these concerns, no conclusive adverse effects have been observed as a result of CLA inclusion in the diet. For instance, it has been suggested that the effect of CLA on glucose homeostasis and insulin resistance may be temporary due to changes in lipid metabolism. Additionally, while some reports show that CLA may increase oxidative stress markers, the mechanism of action is not well understood, whereas many other studies have shown a decrease in not only oxidative stress markers but anti-inflammatories as well (Kim et al, 2016; Viladomiu et al., 2016). Basirico et al. (2015) showed the ability of CLA supplementation to act as an antioxidant, protecting against oxidative stress by increasing nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione (GSH) availability without inducing the production of reactive oxygen species (ROS). CLA has the potential to act much like dietary omega-3 fatty

acids when supplemented, which have been shown to benefit the health of many species (Hall et al., 2004; King et al., 2008).

Previous investigation on CLA supplementation has shown that it elicits many beneficial physiological properties, including acting as an anticarcinogenic and antiadipogenic agent as well as affecting bone formation and lipid metabolism (Belury, 2002; Derakhshande-Rishehri et al., 2014; Eftekhari et al., 2013; Kim et al., 2016; Koba and Yanagita, 2014; O'Quinn et al., 2000; Ostrowska et al., 1999; Pariza, 2004). The anticarcinogenic effect of CLA has been evaluated in many experimental animals, particularly in rats and mice. Several studies reviewed by Belury (2002) have shown significant reduction in the development of mammary, prostate, and skin carcinogenesis when animals were fed a diet supplemented with CLA, though some studies show inconsistent results. CLA is believed to inhibit both the initiation stage of carcinogenesis as well as tumor promotion, although its role has not yet been fully elucidated. Potential mechanisms include modulating free radical oxidation of cells, carcinogen metabolism, carcinogen-DNA adduct formation, and the induction of apoptosis (Belury, 2002). In postmenopausal women, there is evidence of CLA exhibiting protective effects against estrogen-dependent types of breast cancer by regulating estrogen receptor (ER) signaling pathways, inhibiting cancer cell growth, and increasing apoptosis in ER-positive breast cancer cells (Kim et al., 2016). CLA may also exert an anticarcinogenic effect is through the activation of peroxisome proliferator-activated receptors (PPARs). PPARs are transcription factors that regulate the expression of genes involved in energy regulation, glucose homeostasis, and immune function (Viladomiu et al., 2016). PPARγ, for instance, has been shown to induce apoptosis, while both PPARα and PPARγ inhibit the

proliferation of several types of cancer by inhibiting nuclear factor (NF)-κB and activator protein 1 (AP1) activation in cancerous cells (Koba and Yanagita, 2014). CLA may be incredibly beneficial for cancer patients when used in conjunction with other current treatments, especially since the anti-inflammatory properties of CLA may also serve to prevent the increased inflammatory responses associated with radiotherapy.

While not much CLA research has been directed towards horses, there is much potential within the equine industry for CLA use that warrants further exploration. One of the benefits that would be immensely useful in the equine industry is the ability of CLA to reduce the accumulation of adipose tissue. This has already been demonstrated in several species, such as pigs, rats, mice, and humans. Several studies have shown that supplementing CLA to the diet improves carcass quality by increasing the rate and efficiency of gain, reducing fat deposition, and increasing lean tissue in swine. These changes not only improve the quality of meat produced but also help produce less waste by increasing yield and decreasing the cost of production due to increased average daily gain (O'Quinn et al., 2000; Ostrowska et al., 1999; Thiel-Cooper et al., 2001; Zhang et al., 2016). A similar effect has been reported in rats, where the improvement of liver function and modification of lipid metabolism in insulin-resistant rats was seen when CLA was supplemented at 1.5% (Chin et al., 1994; Noto et al., 2006). CLA supplementation, particularly the t10, c12-CLA isomer, also inhibits milk fat synthesis in dairy cows, which may cause a shift in energy partitioning towards other milk constituents, like protein and lactose, while preventing the excessive mobilization of lipids. This not only improves the protein yield in the milk but also stabilizes the health of the cow at the onset of lactation (Ghazal et al., 2014; Masur et al., 2016). The effect of

CLA supplementation in humans has been variable, for instance, Madry et al. (2016) reported that CLA did not affect body weight reduction or BMI but did decrease hip circumference. However, it is likely that these effects on adiposity are species dependent or dependent on preexisting adiposity, making the exploration of CLA's effects on different species essential to understanding its potential benefits.

The mechanisms by which CLA reduces adiposity may involve pathways that regulate energy expenditure, energy intake, and gene expression of lipid and muscle metabolism. The body composition changes that occur with CLA supplementation are a result of CLA's effects on adipocyte and muscle metabolism, including enhanced fat utilization in the muscle through β-oxidation and the reduction of lipid storage, synthesis, and adipogenesis in adipocytes (Kim et al., 2016; Lehnen et al., 2015). CLA may also target skeletal muscle metabolism; it has been shown that CLA supplementation increases lean body mass, energy expenditure, and mitochondrial biogenesis through the regulation of AMP-activated protein kinase (AMPK), which subsequently activates PPARγ coactivator-1α (PGC-1α) and PPAR-δ. Increased mitochondrial biogenesis causes a transformation of muscle fibers from glycolytic fast-twitch type IIB to oxidative slow-twitch type I (Kim et al., 2016). Since type I muscle fibers utilize lipids for energy more than type IIB fibers, this shift in muscle fiber type also increases lipolysis in adipocytes to provide an energy source for muscle fibers. CLA is a potent ligand and activator of PPARs, and as such can activate PPARα-regulated lypolytic genes, including carnitine-palmitoyl transferase (CPT), acyl CoA odixase, and uncoupling protein (UCP) (Koba and Yanagita, 2014; Lehnen et al., 2016). An increase in CPT and UCP concentration and activity increases mitochondrial lipolysis in muscle fibers while

simultaneously reducing the fatty acids and triglycerides available for accumulation in adipose and muscle tissue (Kim et al., 2016; Lehnen et al., 2016). The reduction of body fat may also be a result of CLA's regulation of genes through PPARγ inhibition, particularly the genes that control the differentiation of pre-adipocytes to mature adipocytes, which reduces lipogenesis (Lehnen et al., 2016; Masur et al., 2016).

If CLA can be shown to increase lean muscle mass while decreasing fat deposition in horses, it could be of great use within the equine industry, with the potential to increase lean muscle mass in working and performance horses and to help manage horses with equine metabolic syndrome (EMS). Frank et al. (2010) reviewed this disease that links the presence of obesity, insulin resistance, and laminitis to one clinical syndrome affecting both horses and ponies. Additional symptoms include bilateral lameness and cresty neck scores at or above three using the scale detailed in Carter et al. (2009). Because there is no cure for this syndrome, horses with EMS must be strictly managed, lowering the level of non-structural carbohydrates (NSC), digestible energy (DE), and pasture grass accessible to the horse while also increasing their exercise regimen. These dietary restrictions make a high fiber, fat supplemented diet an attractive option for horses with EMS, specifically utilizing CLA for its antiadipogenic capabilities. CLA enhances insulin sensitivity by activating PPARγ, which is widely used as treatment for type 2 diabetes in humans. PPARγ activation increases plasma adiponectin concentrations and causes the translocation of glucose transporter-4 (GLUT4) to the plasma membrane via the activation of 5'-AMP-activated protein kinase (AMPK). This not only helps improve hyperinsulinemia but also increases glucose uptake in skeletal muscle, which may help normalize glucose metabolism in insulin resistant individuals

(Cho et al., 2016; Koba and Yanagita, 2014). CLA has also been shown to significantly decrease LDL levels and may also lower total cholesterol and triacylglycerol (TAG) levels while increasing HDL concentrations. It has been suggested that CLA decreases LDL levels by inhibiting the secretion of apolipoprotein B (apo B) or by increasing the clearance rate of circulating LDLs through increased LDL receptor activity. CLA may also decrease TAG levels by inhibiting the expression of hepatic stearoyl-CoA desaturase, which is an enzyme involved in TAG synthesis (Derakhshande-Rishehri et al., 2014).

The anti-inflammatory effect of CLA has been reported in several animal models and would also be immensely beneficial to the equine industry if it is shown to be effective in horses. It is believed that CLA can modify the immune response by regulating the production of soluble factors and inflammatory molecules. Both major isomers of CLA have been shown to decrease immune responses by lowering the activity of monocytes, macrophages, dendritic cells and natural killer cells while also decreasing the production of prostaglandins and leukotrienes (Viladomiu et al., 2016). CLA also increases the production of IgG, IgM, and IgA while suppressing IgE, IL-12, and PGE2 expression, all of which contribute to airway inflammation during an allergic reaction (Jaudszus et al., 2016; Kim et al., 2016; Viladomiu et al., 2016). CLA can act as a modulator of T-cell responses by enhancing the cytotoxic potential of blood lymphocytes and proliferation of TCRαβCD8αα cells, both of which help modulate the phenotype and functions of CD8+ cells that are involved with both adaptive and innate immunity. Furthermore, CLA is able to alter the differentiation of monocytes to macrophages, which controls the subsequent activation and differentiation of T- and B-cells (Viladomiu et al.,

2016). Another way in which CLA effects the immune response is through modulating cytokine expression. CLA can diminish the production or activation of pro-inflammatory cytokines, such as IL-6, TNFα, IFNγ, IL-1β, and nuclear factor kappa B (NF-κB). One way that CLA is able to regulate cytokine production is through PPARs. When activated by CLA, PPARs like PPARγ inhibit the transcription of these pro-inflammatory cytokines (Eftekhari et al., 2013; Kim et al., 2016; Viladomiu et al., 2016). Through PPAR and retinoic acid X receptor (RXR), CLA may also combat oxidative stress caused by ROS formation when supplemented at a rate of 1-2% of the diet (Moraes et al., 2016). CLA may be able to prevent an inflammatory response by inhibiting inflammatory prostaglandin (PG) production, and subsequent PGE_2 and $PGF_{2\alpha}$ production, through a NF-κB dependent signaling mechanism. For instance, Badinga et al. (2016) reported that CLA was able to decrease lipopolysaccharide (LPS)-induced release of PG and hypothesized that CLA may act by preventing nuclear translocation of NF-κB.

Because there are considerable differences in the effect of CLA on different species, there is still much about the effects of CLA that is unknown, especially in the horse. Previous work with horses has reported lower concentrations of inflammatory agent collagenase cleavage neopeptide (C2C) and higher concentrations of carboxypeptide of type II collagen (CPII), an anti-inflammatory agent. This suggests that supplementing CLA can help stimulate cartilage synthesis and mitigate degradation of the joints after an inflammation challenge (Bradbery et al., 2014). This work is consistent with literature reviewed by Viladomiu et al. (2016), showing the ability of CLA to moderate inflammation responses. Headley et al. (2012) showed that supplementing CLA to horse diets can alter circulating fatty acid levels, decreasing plasma arachidonic acid

concentrations as well as serum triglycerides and total cholesterol. However, they did not observe any changes in body condition or body weight as a result of supplementation at a rate of 0.30% of daily diet. Because they relied on a crossover design, Headley et al. (2012) recommended that further research involving longer supplementation and withdrawal periods may help clarify some of their results and eliminate the treatment x day interaction they experienced during their crossover experiment. Elevating the level of CLA supplementation could also further clarify any effect CLA may have on body fat deposition and anti-inflammatory properties in horses. If CLA can be shown to elicit positive anti-inflammatory effects in horses, it would be immensely beneficial within the equine industry, particularly regarding competition or performance horses that undergo elevated levels of stress.

Several different methods have been developed to determine fat accumulation and muscle mass or thickness in horses. The most widely accepted and utilized method for determining body fat and overall condition in horses is the Henneke (1983) body condition scoring system. This subjective method utilizes a scale from one to nine – one being extremely thin and nine being extremely obese – assessed both visually and by palpation over six major points of fat accumulation on the body. Similarly, the crest scoring system is another subjective method of measuring body fat using a scale from zero to five – zero being no neck crest visible or palpable and five being a neck crest so large it droops permanently to one side (Carter et al., 2009). While these methods are more commonly used by the equine industry, they do not provide an objective means of evaluating body fat and overall condition. One noninvasive way of quantifying body condition involves taking transdermal ultrasound imaging of different muscle groups to

measure both muscle thickness, cross-sectional area, and rump fat thickness (Linder et al., 2010). While ultrasounds do provide a means of objectively quantifying muscle and fat composition, they are not necessarily a practical or readily available means of measurement to the average horse owner.

CHAPTER III

Objectives

The purpose of this investigation was divided into multiple phases:

Phase I: Determine the maximum inclusion rate of supplemental CLA to young developing horses.

Phase II: Evaluate the effects of supplementing elevated levels of CLA on growth performance, lean muscle mass, and fat deposition.

CHAPTER IV

Materials and Methods

All experimental procedures were approved by the Sam Houston State University (SHSU) Institutional Animal Care and Use Committee prior to the start of the experiment (16-09-08-1008-3-01). Horses were housed at the SHSU Agricultural Center in 3 x 3 m individual stalls for the duration of this study and were used in conjunction with the Equine Behavior and Training courses (EQSC 3340 Equine Behavior and Training I; EQSC 4391 Equine Behavior and Training II).

Phase I: Determining Palatability

Ten Quarter horses (2-6 yrs, 435.53 kg) from private owners were utilized to determine the highest level of CLA supplementation that is palatable to horses. Horses were fed at 1% of body weight (BW) daily of a commercially available pelleted concentrate (SafeChoice, Cargill Animal Nutrition, Elk River, MN) and approximately 1% BW of coastal bermudagrass hay divided equally between two meals. Diets were formulated to meet or exceed all NRC (2007) nutritional requirements for mature horses performing light exercise. Soybean oil is the most readily available and widely utilized by the equine industry as a fat supplement, and as such was utilized to provide a baseline level of fatty acid palatability in an equine model. Soybean oil was topdressed onto feed and mixed immediately prior to each meal beginning at 5.0% per day of the total concentrate diet. Every three days, horses were weighed before their morning feeding, and the level of oil supplementation was increased if no significant refusals were observed during the previous feeding period. Once significant refusals were discerned, the level of supplementation was decreased until palatability returned. For each meal,

horses were given one hour to eat before refusals were collected and weighed. Once a supplementation level was discerned with the soybean oil, CLA (BASF Corp., Florham Park, NJ) was substituted for soybean oil to ensure palatability was the same across treatments. The same protocol for supplementation rate and feeding were applied for both soybean oil and CLA supplementation. Changes in intake levels across the different oils and levels of supplementation were analyzed using the PROC GLM procedure in SAS v. 9.4 (SAS Inst. Inc., Cary, NC). Effects were considered significant when $P \leq 0.05$.

Phase II: CLA Supplementation

Nine stock-type horses (1-3 yrs, 384.45 kg) from private owners were utilized in a 12-week completely randomized design where horses were randomly assigned to treatment groups of either CLA (BASF Corp., Florham Park, NJ) or soybean oil control (n = 5 and 4/group, respectively) with age, BW, and sex evenly distributed across treatments. Horses were fed at 1% BW daily of a commercially available pelleted concentrate (SafeChoice, Cargill Animal Nutrition, Elk River, MN) and approximately 1% BW of coastal bermudagrass hay divided equally between two meals. Diets met or exceeded all NRC (2007) nutritional requirements for mature horses performing light exercise. Both control and treatment groups were supplemented at 1.5% of the total concentrate diet (0.015% BW) and were topdressed onto feed and mixed immediately prior to each meal. Diet adaptation took place over a 12 d period prior to the start of the trial, with the first 6 d being adaptation to the concentrate feed and to ensure all horses begin the trial on a consistent plane of nutrition. Oil supplementation began 6 d prior to the beginning of the study in order to acclimate horses to the treatment. They received 25% of total treatment dose on the first day of oil supplementation and was increased

every 2 d by 25% so that they received their full ration on d 0.

Starting on d 0, data collection entailed weekly body weights (BW) on fasted horses as well as body condition score (BCS), heart girth, crest circumference, and crest score to assess body composition and to reformulate diets while blood was collected biweekly. Heart girth and crest circumference were measured with a soft tape measure placed around the heart girth and at the approximate center of the neck where a horse would have the greatest crest circumference. A single, trained individual determined BCS and crest score each week utilizing the scales outlined in Henneke (1983) and Carter et al. (2009), respectively. Rump fat thickness (RFT), ribeye area (REA), back fat thickness (BF), and intramuscular fat (IMF) measurements were conducted via transdermal ultrasonography by the same centralized ultrasound processing (CUP) certified technician using an ultrasound system (Aloka SSD-500V, Aloka Inc., Tokyo, Japan) in conjunction with a 3.5 MHz 172 mm linear transducer. Measurements were taken on d 0, midway through the trial period (d 42), and at the completion of the trial (d 84). Images were analyzed by Designer Genes Technologies (Harrison, AR). REA and BF were obtained via a cross-sectional image between the 13th and 14th ribs (REA13 and BF13, respectively) and between the 17th and 18th ribs (REA17 and BF17, respectively). RFT was measured at 5 cm lateral from the midline halfway between the first coccygeal vertebrae and the ischium (Westervelt et al., 1976). IMF measurements within the longissimus dorsi were obtained by imaging parallel to the backbone between the 17th and 18th ribs. To ensure proper contact between transducer and horse, the transducer was fitted with a PIA contour pad (Animal Ultrasound Services, Ithaca, NY) designed to conform to the curvature of a horse's back. In addition, corn oil was applied to promote

acoustical contact between animal and transducer (Perkins et al., 1992). Changes in growth performance, subjective body composition, and ultrasound measurements were analyzed using PROC MIXED with repeated measures in SAS with the main effects of treatment and time included in the model. Additionally, PROC GLM was used to determine within day effects of treatments. Effects were considered significant when $P \leq 0.05$.

CHAPTER V

Results

Palatability

From phase I of this study, horses were observed to consume no more than 5.0% CLA of the concentrate diet daily without significant refusal (Figure 1) with the range of fatty acid supplementation ranging from 5.0% to 10.0% of the total daily concentrate diet. Soybean oil supplementation exhibited a difference between the 5.0% and 10.0% supplementation levels ($P < 0.05$) but not between the 5.0% and 7.5% supplementation levels ($P = 0.13$). Higher refusals were observed when CLA was supplemented at 7.5% compared to when it was supplemented at 5.0% ($P < 0.01$). At the 5.0% supplementation level, there was no difference between soybean oil and CLA ($P = 0.48$); however, above a 5.0% supplementation level, CLA exhibited higher refusals than soybean oil ($P < 0.01$). For phase II, the inclusion rate was determined at 2.5% of the concentrate diet. However, significant refusals of both treatment oils were observed during the adaptation period of phase II when treatment and control oils were supplemented at a rate above 1.5% of the concentrate diet. Therefore, to ensure palatability throughout the trial period, treatments for phase II of this study were included at 1.5% of the concentrate diet.

Body Composition

On occasion throughout the supplementation period, individual horses in both treatment groups did not consume the entirety of their meals. However, daily intake did not differ across treatments ($P > 0.05$) at any time throughout the trial (Figure 2). In general, body composition measurements including BW, BCS, heart girth, crest score, and crest circumference for horses consuming either CLA or soybean oil (CON) did not

differ across treatments (Table 1). BW and BCS increased over time ($P < 0.01$), but did not exhibit a difference between treatments ($P = 0.17$ and 0.20, respectively) or a treatment x day interaction ($P = 1.00$ and 0.65, respectively). As expected from the increase in BW and BCS, heart girth, crest circumference, and crest score also increased over time ($P < 0.01$). Neither crest circumference nor crest score differed across treatments ($P = 0.55$ and 0.82, respectively), nor was there a treatment x day interaction for either variable ($P = 0.25$ and 0.61, respectively). There was, however, a tendency for heart girth to be higher in CLA supplemented horses over time ($P = 0.07$).

Ultrasound

Initial ultrasound measurements were recorded on d 0; however, initial data were lost in a computer crash and were unable to be recovered. As a result, initial measurements were retaken on d 7. Similar to the changes observed in growth performance, REA13, BF13, REA17, BF17, IFM, and RFT generally did not differ across treatments (Table 2). REA13 and BF13 increased over time ($P < 0.01$ and 0.05, respectively) but did not exhibit a treatment x day interaction ($P = 0.69$ and 0.95, respectively) or differ across treatments ($P = 0.22$ and 0.15, respectively). REA17 exhibited a treatment x day interaction ($P < 0.05$). However, because REA17 in CLA supplemented horses (103.69 $cm^2 \pm 2.364$) was higher than control horses (91.96 $cm^2 \pm 2.643$) on d 7 ($P = 0.04$), REA17 was reanalyzed with d 7 as a covariate for all subsequent measures to account for this initial difference (Figure 3). After accounting for the initial variance, REA17 tended to be higher in treatment horses (100.98 $cm^2 \pm 2.62$) than control horses on d 42 (91.44 $cm^2 \pm 2.994$; $P < 0.07$). Similarly, on d 84, REA17 tended to be higher in treatment horses (106.91 $cm^2 \pm 2.296$) than control horses (98.389

cm^2 ± 2.621; $P < 0.07$). BF17 tended to be different over time ($P = 0.07$) but did not exhibit a difference across treatments ($P = 0.30$) or a treatment x day interaction ($P = 0.88$). IMF did not differ over time ($P = 0.86$), across treatments ($P = 0.96$) or a treatment x day interaction ($P = 0.55$). RFT increased over time ($P < 0.01$), but did not differ across treatments ($P = 0.51$) and did not exhibit a treatment x day interaction ($P = 0.70$).

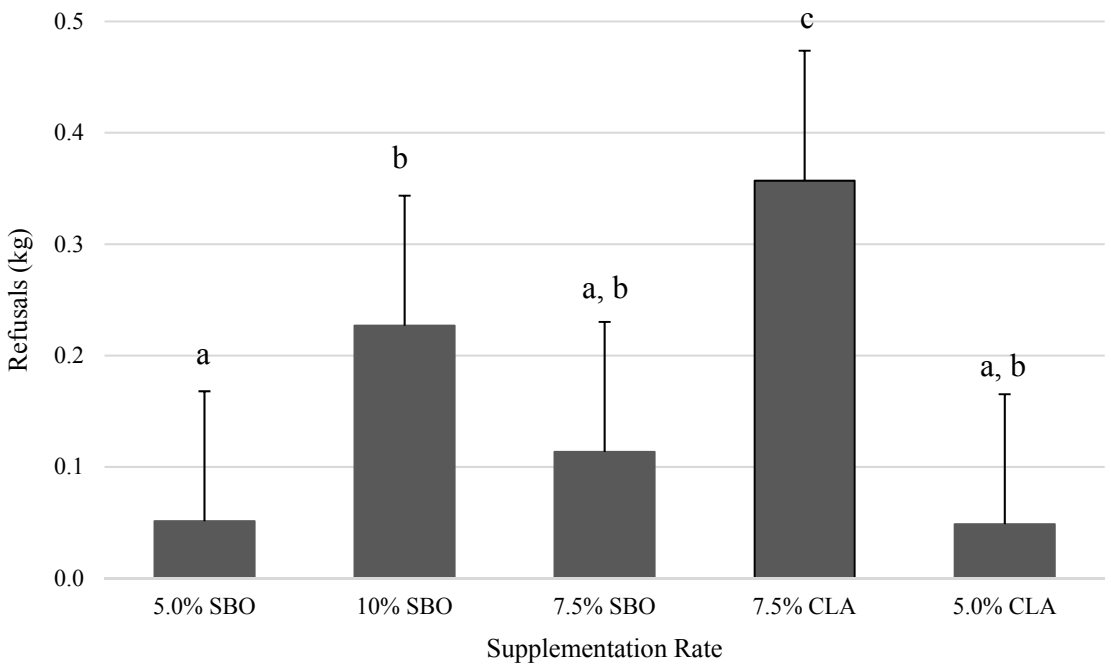

Figure 1. Phase I Refusals. Mean refusals (kg) for each level of supplementation during the palatability phase of this study. Supplementation rate is given as a percent of total daily concentrate diet of either soybean oil (SBO) or conjugated linoleic acid (CLA). Pooled Standard error of the treatment mean was used as an indication of variation. Means with different superscripts differ at $P \leq 0.05$.

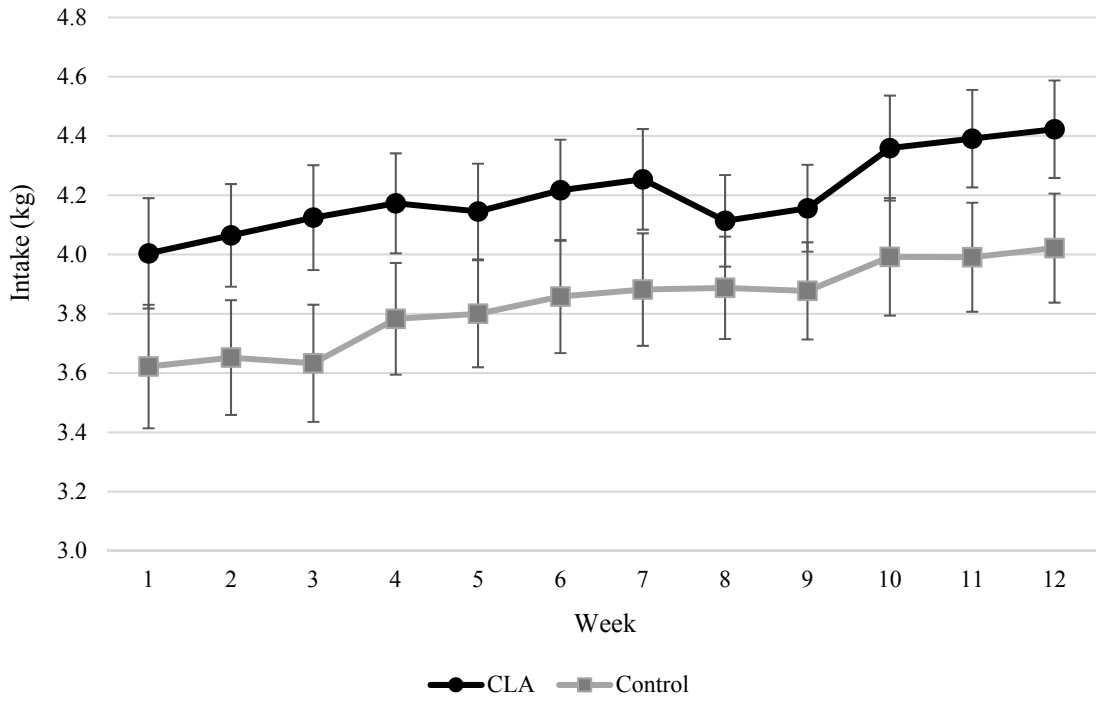

Figure 2. Phase II Intake. Mean daily intake (kg) per week for both control and treatment groups during phase II. Intake did not differ across treatments at any time throughout the trial ($P > 0.05$). Pooled Standard error of the treatment mean was used as an indication of variation.

Table 1

Mean BW (kg), BCS, heart girth (cm), crest score, and crest circumference (cm) for horses consuming either soybean oil (CON) or CLA[a].

	Mean			*P*-Values[b]		
	CON (n=4)	CLA (n=5)	SE[c]	Trt	Day	Trt * Day
BW (kg)	385.74	424.82	12.872	0.17	< 0.01	1.00
BCS	5.50	5.88	0.151	0.20	< 0.01	0.65
Heart Girth (cm)	169.03	173.22	2.132	0.34	< 0.01	0.07
Crest Score	1.73	2.03	0.304	0.61	< 0.01	0.82
Crest Circumference (cm)	87.07	90.78	1.728	0.25	< 0.01	0.55

[a] – Conjugated linoleic acid supplement manufactured by BASF Corp. (Florham Park, NJ).
[b] – *P*-values for main effects of treatment, day, and the interaction of treatment and day.
[c] – Pooled Standard error of the treatment mean.

Table 2

Mean ultrasound measurements for both soybean oil (CON) and treatment (CLA[c]) groups on d 7, 42, and 84.

Day	7				42[d]				84[d]			
	CON (n=4)	CLA (n=5)	SE[e]	*P*-Value	CON (n=4)	CLA (n=5)	SE[e]	*P*-Value	CON (n=4)	CLA (n=5)	SE[e]	*P*-Value
REA17[f] (cm^2)	90.08[a]	99.94[b]	4.459	0.04	91.44	100.98	4.459	0.07	98.39	106.91	4.459	0.07
BF17[g] (cm)	0.37	0.45	0.0308	0.30	0.29	0.34	0.0308	0.54	0.43	0.46	0.0308	0.70
REA13[h] (cm^2)	82.26	85.86	0.6212	0.38	77.53	83.34	0.6212	0.16	91.13	92.34	0.6212	0.77
BF13[i] (cm)	0.42	0.59	0.0503	0.21	0.32	0.48	0.0503	0.23	0.47	0.67	0.0503	0.15
IMF[j] (%)	3.54	3.73	0.3264	0.56	3.77	3.65	0.3264	0.72	3.72	3.69	0.3264	0.92
RFT[k] (cm)	0.55	0.76	0.1148	0.50	0.81	0.94	0.1148	0.66	0.87	0.86	0.1171	0.43

[a-b] – Within day, means with different superscripts differ at $P \leq 0.05$.
[c] – Conjugated linoleic acid supplement manufactured by BASF Corp. (Florham Park, NJ).
[d] – The initial difference in REA17 on d 7 was accounted for by re-analyzing d 42 and 84 with d 7 as a covariate.
[e] – Pooled Standard error of the treatment mean.
[f] – Ribeye area between the 17th and 18th ribs in cm^2.
[g] – Fat content between the 17th and 18th ribs in cm.
[h] – Ribeye area between the 13th and 14th ribs in cm^2.
[i] – Fat content between the 13th and 14th ribs in cm.
[j] – Percent intramuscular fat.
[k] – Rump fat thickness in cm.

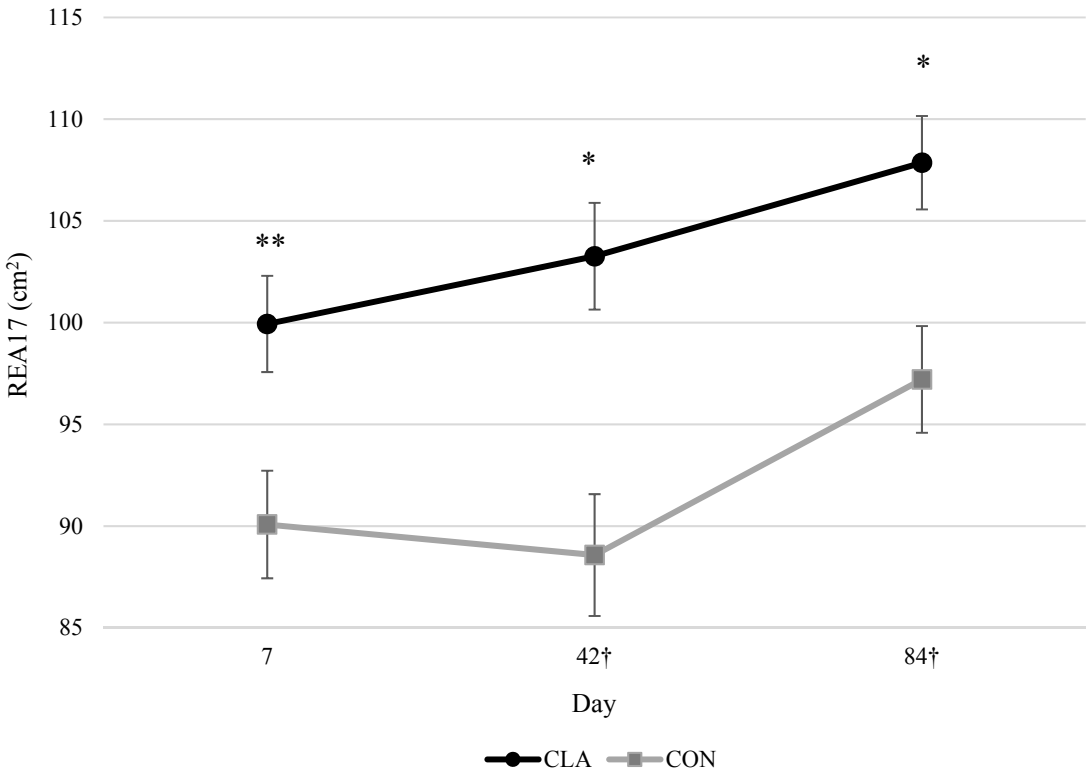

Figure 3. Ribeye area between the 17th and 18th ribs (REA17). Changes in REA17 between treatments over time. The initial difference in REA17 on d 7 was accounted for by re-analyzing d 42 and 84 with d 0 as a covariate, as signified by †. Within day, a double asterisk (**) signifies that treatments differ at $P \leq 0.05$ while a single asterisk (*) signifies a tendency for treatments to differ at $P < 0.07$. Pooled Standard error of the treatment mean was used as an indication of variation.

CHAPTER VI

Discussion

Fat supplementation has long been utilized to improve the health and performance of animals, including horses. Dietary omega-3 fatty acids have been shown to elicit several health benefits, including improved immune function and overall health (King et al., 2008). One potential downfall of supplementing n-3 fatty acids like EPA and DHA is that they are frequently marine-derived, which can create palatability issues. CLA is a potentially more palatable alternative to dietary n-3 fatty acids that may offer benefits like that of n-3 fatty acids, such as reduction of body fat content and increase of lean muscle mass (O'Quinn et al., 2000; Ostrowska et al., 1999). The purpose of this study was to investigate if elevated dietary CLA would alter lean muscle mass and fat deposition in young, developing horses. Data from previous equine studies have shown that CLA can affect plasma fatty acid levels when compared to a control oil. Headley et al. (2012) utilized corn oil as a control, but soybean oil was utilized in this study as it is the most readily available and is a fat supplement widely used in the equine industry to finish out performance horses.

Palatability

To our knowledge, the supplementation rate of CLA in previous equine studies has not exceeded 1.0% of total diet. Supplementing CLA at 1.0% of total diet increased ADG and decreased fat content in hogs (Thiel-Cooper et al., 2001), but did not exhibit the same change in body composition in horses at a lower inclusion rate (Headley et al., 2012), which suggests a higher level of supplementation may be required to achieve results similar to that of other species. Results from the initial phase of this study

indicated that refusals increased significantly when CLA was supplemented above 5.0% of the total daily concentrate diet, which suggests that supplementing at or below that level would prevent a reduction in feed intake levels. However, during phase II CLA could not be supplemented above 1.5% of total diet without significant refusals. This could possibly be the result of individual horse preference, some horses during either phase of this study would consume their concentrate without any added oil but would stop eating completely when supplemented with CLA, while others never had any refusals no matter how much CLA was supplemented. Horses that stopped eating completely upon beginning CLA supplementation were removed from the study before d 0. Further study with greater numbers is needed to clarify the reason for the conflicting results and to clarify the maximum possible inclusion rate of CLA supplementation in horses.

Body Composition

Results of this study indicate that CLA supplementation does not influence BW, BCS, or other growth performance measurements like heart girth and crest circumference. This coincides with results of Headley et al. (2012), who did not find an effect of CLA on body composition. While previous literature has shown that CLA exhibits an anti-adipogenic effect on body composition in numerous species including swine, rats, and humans (Ostrowska et al., 1999; O'Quinn et al., 2000; Thiel-Cooper et al., 2001), the same effect has not been successfully seen in the horse. The effect of CLA on body composition has been shown to be quite variable across species, so it is possible that CLA does not exert the same anti-adipogenic effect in equines as seen in other species. In contrast to data seen in hogs, CLA supplementation in mature women did not

alter BW or BMI but did decrease hip circumference (Madry et al., 2016). Alternatively, it is possible that the effect of CLA on body composition is dependent on pre-existing adiposity, and as such may be more evident in obese or insulin-resistant horses than in young, developing horses. For instance, both Chin et al. (1994) and Noto et al. (2006) reported a significant improvement in lipid metabolism in insulin-resistant rats supplemented with CLA. In this study, there was a tendency for heart girth to be higher in CLA supplemented horses over time, so there is still potential for CLA to affect body composition. Future studies with prolonged CLA supplementation periods are needed to help clarify the effect of CLA on equine body composition.

Ultrasound

Data from the current study indicates that CLA does not affect body fat content, including RFT, IMF, BF13, and BF17. These results are in contrast with previous literature that demonstrated t10, c12-CLA supplementation alters body composition by decreasing body fat content in finishing swine (Ostrowska et al., 1999; O'Quinn et al., 2000; Thiel-Cooper et al., 2001). This difference may be the result of a species-dependent effect of CLA supplementation, but results from the current study also contradict data from Headley et al. (2012) that indicated a tendency for CLA to increase RFT in horses. Alternatively, if the effect of CLA is dependent upon pre-existing adiposity, then this difference may be the result of supplementing CLA to young, developing horses as opposed to mature horses with higher fat content. Further study is needed to clarity the reason for the conflicting results.

While data from this study do not indicate a difference in REA13 between treatment groups, there was a tendency for REA17 to be higher in CLA supplemented

horses. These data coincide with results of previous literature demonstrating an increase in lean muscle mass because of CLA supplementation (O'Quinn et al., 2000; Ostrowska et al., 1999; Thiel-Cooper et al., 2001). However, the mechanism of action controlling this increase remains unclear. An increase in lean muscle mass due to CLA supplementation contradicts previous study demonstrating a shift in muscle fiber type from type IIB to type I as a result of CLA supplementation, which would cause the muscle fiber to shrink in size (Kim et al., 2016). It would be useful to take muscle biopsies in addition to ultrasound measurements to track any changes in muscle fiber type over the supplementation period to clarify the effect of CLA on lean muscle mass. Additionally, while several studies have shown that dietary CLA is absorbed and incorporated into circulation as early as 2 to 6-wk of supplementation (Headley et al., 2012; King et al., 2008), the incorporation into tissues inevitably takes longer and changes to the tissue longer still. Plasma fatty acid levels were not able to be analyzed for this study, but would be a beneficial addition to track changes over the supplementation period and to help determine how long it will take CLA to incorporate into tissues. As such, it is possible that a longer supplementation period will be necessary to demonstrate a difference in both fat content and lean muscle mass between control oils and CLA.

CHAPTER VII

Conclusions

While the inclusion rate of this study was higher than previous studies, the maximum inclusion rate of CLA in the equine diet remains unclear in an equine model after the two phases of this study. Dietary supplementation of CLA at 0.015% BW over a 12-wk feeding period tended to increase lean muscle mass but did not affect overall growth performance or body fat content. Results of this current study indicate that CLA may be a beneficial supplement for performance horses that require more muscle mass. It would be beneficial to include muscle biopsies and plasma fatty acid analysis in future studies to further clarify the necessary length of supplementation and in order to see any potential effect of CLA on changes in muscle fiber type and lean muscle mass. Further study to determine the most appropriate inclusion rate in the equine diet may also help clarify the effect of CLA on body composition, including fat content, which may have implications for horses suffering from EMS.

REFERENCES

Aloka Inc. 2016. Tokyo, Japan.

Animal Ultrasound Services. 2016. Ithaca, NY.

Badinga, L., M. S. Gulay, and A. Ealy. 2016. CLA and EPA inhibit LPS-induced prostaglandin release from bovine endometrial cells through an NF-κB-dependent signaling mechanism. Turk. J. Vet. Anim. Sci. 40:382-388.

BASF Corporation. 2016. Florham Park, NJ.

Basirico, L., P. Morera, D. Dipasquale, A. Troscher, A. Serra, M. Mele, and U. Bernabucci. 2015. Conjugated linoleic acid isomers strongly improve the redox status of bovine mammary epithelial cells (BME-UV1). J. Dairy Sci. 98:7071-7082.

Belury, M.A. 2002. Dietary conjugated linoleic acid in health: physiological effects and mechanisms of action. Annu. Rev. Nutr. 22:505-531.

Bradbery, A.N., J.A. Coverdale, K.L. Vernon, J.L. Leatherwood, C.E. Arnold, R.A. Dabareiner, M.K. Kahn, A.A. Millican, T.H. Welsh, and S.B. Smith. 2014. Evaluation of conjugated linoleic acid supplementation on markers of joint inflammation and cartilage metabolism in young horses challenged with lipopolysaccharide. Master's thesis, Texas A&M University.

Cargill Animal Nutrition. 2016. Elk River, MN.

Carter, R.A., R.J. Geor, W. B. Staniar, T.A. Cubitt, and P.A. Harris. 2009. Apparent adiposity assessed by standardized scoring systems and morphometric measurements in horses and ponies. Vet. J. 179:204-210.

Chin, S.F., J.M. Storkson, K.J. Albright, M.E. Cook, and M.W. Pariza. 1994. Conjugated linoleic acid is a growth factor for rats as shown by enhanced weight gain and improved feed efficiency. J. Nutr. 124:2344-2349.

Cho, K., Y. Song, and D. Kwon. 2016. Conjugated linoleic acid supplementation nhances insulin sensitivity and peroxisome proliferator-activated receptor gamma and glucose transporter type 4 protein expression in the skeletal muscles of rats during endurance exercise. Iran. J. Basic Med. Sci. 19:20-27.

Derakhshande-Rishehri, S., M. Mansourian, R. Kelishadi, and M. Heidari-Beni. 2014. Association of foods enriched in conjugated linoleic acid (CLA) and CLA supplements with lipid profile in human studies: a systematic review and meta-analysis. Public Health Nutr. 18(11):2041-2054.

Designer Gene Technologies. 2016. Harrison, AR.

Eftekhari, M.H., F. Aliasghari, M.A. Babaei-Beigi, and J. Hasanzadeh. 2013. Effect of conjugated linoleic acid and omega-3 fatty acid supplementation on inflammatory and oxidative stress markers in atherosclerotic patients. Mediterr. Health J. 14(2):305-313.

Frank, N., R.J. Geor, S.R. Bailey, A.E. Durham, and P.J. Johnson. 2010. Equine metabolic syndrome. J. Vet. Intern. Med. 24:467-475.

Ghazal, S., V. Berthelot, N.C. Friggens, and P. Schmidely. 2014. Effects of conjugated linoleic acid supplementation and feeding level on dairy performance, milk fatty acid composition, and body fat changes in mid-lactation goats. J. Dairy Sci. 97:7162-7174.

Hall, J.A., R.J. Van Saun, and R.C. Wander. 2004. Dietary (n-3) fatty acids from menhaden fish oil alter plasma fatty acids and leukotriene B synthesis in healthy horses. J. Vet. Intern. Med. 18:871-879.

Headley, S., J.A. Coverdale, T.C. Jenkins, C.M. Klein, J.L. Sharp, and K.L. Vernon. 2012. Dietary supplementation of conjugated linoleic acid in horses increases plasma conjugated linoleic acid and decreases plasma arachidonic acid but does not alter body fat. J. Anim. Sci. 90:4876-4882.

Henneke, D., G. Potter, J. Kreider, and B. Yeates. 1983. Relationship between condition score, physical measurements and body fat percentage in mares. Equine Vet. J. 15:371-372.

Jaudszus, A., J.G. Mainz, S. Pittag, S. Dornaus, C. Dopfer, A. Roth, and G. Jahreis. 2016. Effects of a dietary intervention with conjugated linoleic acid on immunological and metabolic parameters in children and adolescents with allergic asthma – a placebo-controlled pilot trial. Lipids Health Dis. 15:21.

Kim, J.H., Y. Kim, Y.J. Kim, and Y. Park. 2016. Conjugated linoleic acid: Potential health benefits as a functional food ingredient. Ann. Rev. Food Sci. Technol. 7:221-244.

King, S.S., A.A. AbuGhazeleh, S.K. Webel, and K.L. Jones. 2008. Circulating fatty acid profiles in response to three levels of dietary omega-3 fatty acid supplementation in horses. J. Anim. Sci. 86:1114-1123.

Koba, K. and T. Yanagita. 2014. Health benefits of conjugated linoleic acid (CLA). Obes. Res. Clin. Pract. 8:e525-e532.

Lehnen, T.E., M.R. da Silva, A. Camacho, A. Marcadenti, and A.M. Lehnen. 2015. A review on effects of conjugated linoleic fatty acid (CLA) upon body composition and energetic metabolism. J. Int. Soc. Sports Nutr. 12:36.

Linder, A., R. Signorini, J. Vassallo, F. Tomatis, F.M. Flores, M.E. Gagliano, J. Curiotti, and E. Terragona. 2010. Reproducibility and repeatability of equine muscle thickness measurements with ultrasound. J. Equine Vet. Sci. 30(11):635-640.

Madry, E., I. Chudzicka-Strugala, K. Grabanska-Martynska, K. Malikowska, P. Grebowiec, A. Lisowska, P. Bogdanski, and J. Walkowiak. 2016. Twelve weeks CLA supplementation decreases the hip circumference in overweight and obese women. A double-blind, randomized, placebo-controlled trial. Acta Sci. Pol. Technol. Aliment. 15(1):107-113.

Masur, F., F. Benesch, H. Pfannkuche, H. Fuhrmann, and G. Gabel. 2016. Conjugated linoleic acids influence fatty acid metabolism in ovine ruminal epithelial cells. J. Dairy Sci. 99:3081-3095.

Moraes, M.L., A.M.L. Ribeiro, E. Santin, and K.C. Klasing. 2016. Effects of conjugated linoleic acid and lutein on the growth performance and immune response of broiler chickens. Poult. Sci. 95:237-246.

Noto, A., P. Zahradka, N. Yurkova, X. Xie, E. Nitschmann, M. Ogborn, and C.G. Taylor. 2006. Conjugated linoleic acid reduces hepatic steatosis, improves liver function, and favorably modifies lipid metabolism in obese insulin-resistant rats. Lipids. 41(2):179-188.

NRC. 2007. Nutrient Requirements of Horses. 6th rev. Ed. Natl. Acad. Press, Washington, DC.

O'Quinn, P.R., J.L. Nelssen, R.D. Goodband, J.A. Unruh, J.C. Woodworth, J.S. Smith, and M.D. Tokach. 2000. Effects of modified tall oil versus a commercial source of conjugated linoleic acid and increasing levels of modified tall oil on growth performance and carcass characteristics of growing-finishing pigs. J. Anim. Sci. 78:2359-2368.

Ostrowska, E., M. Muralitharan, R.F. Cross, and D.E. Bauman. 1999. Dietary conjugated linoleic acids increase lean tissue and decrease fat deposition in growing pigs. J. Nutr. 129:2037-2042.

Pariza, M.W. 2004. Perspective on the safety and effectiveness of conjugated linoleic acid. Am. J. Clin. Nutr. 79(suppl):1132S-1136S.

Perkins, T.L., R.D. Green, and M.F. Miller. 1992. Evaluation of alternative ultrasound measurement sites as estimators of yield grade factors in beef cattle. Proc. Western Sect. Am. Soc. Anim. Sci. 43:294-297.

Statistical, Analysis Systems Institute. 2000. SAS/STAT User's Guide, Version 9.4. SAS Institute Inc, Cary, NC.

Thiel-Cooper, R.L., F.C. Parrish, Jr., J.C. Sparks, B.R. Wiegand, and R.C. Ewan. 2001. Conjugated linoleic acid changes swine performance and carcass composition. J. Anim. Sci. 79:1821-1828.

Viladomiu, M., R. Hontecillas, and J. Bassaganya-Riera. 2016. Modulation of inflammation and immunity by dietary conjugated linoleic acid. Eur. J. Pharmacol. 785:87-95.

Wang, T. and H.G. Lee. 2013. Advances in research on cis-9, trans-11 conjugated linoleic acid: A major functional conjugated linoleic acid isomer. Crit. Rev. Food Sci. Nutr. 55(5):720-731.

Westervelt, R.G., J.R. Stouffer, H.F. Hintz, and H.F. Schryver. 1976. Estimating fatness in horses and ponies. J. Anim. Sci. 43(4):781-785.

Zhang, H., X. Dong, Z. Wang, A. Zhou, Q. Peng, H. Zou, B. Xue, and L. Wang. 2016. Dietary conjugated linoleic acids increase intramuscular fat deposition and decrease subcutaneous fat deposition in yellow breed x simmental cattle. Anim. Sci. J. 87:517-524.

APPENDIX

Sam Houston State University
A Member of the Texas State University

Institutional Animal Care and Use Committee

Committee Members

Regular Members	Alternate Members
Marcy Beverly, Ph.D.	Kyle Stutts, Ph.D.
James Harper, Ph.D.	Todd Primm, Ph.D.
Mark Anderson, Ph.D.	Ilona Petrikovics, Ph.D.
Autumn Smith-Herron, Ph. D.	Jeff Wozniak, Ph.D.
T.C. Sim, Ph.D.	Michael Moore, D.V.M.
Gerald Etheredge, D.V.M.	
Vernette Porter, Community Member	

Date: September 14, 2016

To: Elizabeth Miller [Faculty Supervisor: Dr. Marcy Beverly]
ASET
Box 2088
Campus

From: Dr. Marcy Beverly, IACUC Chair

Re: **Form C**: Research
ID # 16-09-08-1008-3-01
Project Title: *The evaluation of CLA supplementation on fat deposition and lean muscle mass in horses [Student Thesis]*

Species: *Equine*

Start: September 8, 2016 **End:** September 8, 2019

Your **IACUC Initial Review** submission was reviewed and approved under **Designated Member Review** (DMR) procedures by Dr. Mark Anderson on **August 23, 2016** with the following result:

Approved

Annual Review Form Deadline: September 30, 2017

Sam Houston State University is an Equal Opportunity Institution
*Huntsville, Texas 77341-2447 * 936.294.1180 * Fax 936.294.3798*

VITA

EDUCATION

Sam Houston State University (SHSU), Huntsville, TX — May 2017
Master of Science, Agriculture; equine emphasis — GPA: 4.00/4.00

Truman State University (TSU), Kirksville, MO — May 2015
Bachelor of Science, Biology; Summa Cum Laude — GPA: 3.96/4.00

RESEARCH

T.D. Morgan, A.L. Salazar, F.R. Melgar, E.J. Scholljegerdes, C.A. Loest, L.M. White, K. Marchetti, S.A. Soto-Navarro, K.W. Walter, and S.L. Ivey. 2015. Comparison of titanium dioxide vs. chromic oxide as an external marker to estimate fecal output in horses. Western Section American Society of Animal Science Proceedings 66. Ruidoso, New Mexico.

- Took part in total fecal collections every 6 hours during collection period
- Stored samples for later analysis

E.F. Miller, F.R. Melgar, T.D. Morgan, S.L. Ivey, C.L. Loest, L.M. White, and K.W. Walter. 2015. Evaluation of inter-day variation of horses on total fecal collection. J. Anim. Sci. 93, E-suppl 2:340.

- Project and presentation developed as a selected portion of data from study above
- Evaluate variation of dry matter intake, output, and calculation of digestibility
- Highlight importance of adaptation techniques prior to collection period
- Collaborated with peers on data analysis and writing of abstract

S. Heibeck, J. Altman, B. J. Karren, and K. W. Walter. 2015. Effect of herbal liniment on equine back pain over time: a preliminary study. Abstract T75. J. Anim. Sci. 93, E-suppl 2:339.

- Assisted with lameness evaluations for pre-screening of research horses
- Helped with daily application of treatments
- Contributed to weekly thermal image data collection
- Collaborated with peers to ensure accuracy and efficiency

K.O. Short and K.W. Walter. 2015. Effects of Weight Load on Horse Stride Length. Abstract. Truman State University Student Research Conference. Kirksville, Missouri.

- Assisted with horse handling, which consisted of recording body weights, applying weight loads, and trotting horses in hand for video analysis
- Contributed to software and data analysis

TEACHING EXPERIENCE

Graduate Teaching Assistant, SHSU, Huntsville, TX 2016-2017

- Taught Introductory Animal Science and Equine Science lecture and lab
- Assisted Animal Science faculty
- Proctored and graded exams

Teaching Assistant, TSU, Kirksville, MO 2013-Spring 2015

- Assisted professor in supervising and teaching students to correctly and safely ride and work with horses
- Assisted with administering mid-term and final exams, both written and practical

4H Instructor, TSU, Kirksville, MO Spring 2013

- Researched and prepared several presentations for the Adair County 4H veterinary medicine class
- Taught several of the above classes over prepared presentations

Science on Saturday Instructor, TSU, Kirksville, MO Spring 2013

- Prepared and presented a class on various agricultural topics for elementary school age children

Ag in the Classroom, Kirksville Primary School, Kirksville, MO Spring 2012

- Planned and implemented lessons about agriculture and its relevance to a fourth grade class

ANIMAL EXPERIENCE

Kentucky Equine Research (KER), Versailles, KY Summer 2016
Summer Research Intern

- Worked with other interns and KER staff on various equine research projects, including digestibility studies, glycemic response tests, and drug studies
- Learned to work lab equipment, such as a YSI Glucose/Lactate analyzer and high speed equine treadmill
- Completed daily barn chores, such as preparing feed for trials, picking stalls, and exercising horses on the walker

Equestrian Team, TSU, Kirksville, MO 2011-2015
Treasurer: 2013 President: 2014 Hunt Team Captain: 2015

- Organized and maintained balances for each member of the team as well as bills for the team's competitions
- Presided over all team meetings
- Worked in conjunction with the Funds Allotment Council to request funding for team purposes
- Competed at Intercollegiate Horse Show Association competitions, advancing to Regionals, Zones, and Nationals as an individual Open rider and Cacchione Cup competitor

ANIMAL EXPERIENCE (continued)

Veterinary Technician, Various locations 2011-2013

- Handled and restrained animals (dogs, cats, cattle, and horses) during appointments, pre- and post-surgical procedures
- Performed procedures such as trimming nails, running heart worm tests, weighing animals, staining cytology slides, preparing fecal slides, reading fecal slides, checking vitals, and developing x-rays
- Maintained a sanitary environment by shaving and scrubbing animals pre-surgery, cleaning tables and equipment
- Administered vaccines, pre- and post-surgical drugs, fluids, and flea treatments

Cattle Team, TSU, Kirksville, MO 2012-2013

- Contributed to herd health by castrating, de-worming, vaccinating, and tattooing a portion of the calves each year
- Worked with other students to halter-train heifers in preparation for various beef expos

Parawild Training Course, Hoedspriut, South Africa May 2013

- Part of Large Mammal Conservation study abroad course
- Learned how to handle, capture, and care for various wild animals for relocation
- Treated minor injuries sustained by wild animals during capture
- Emphasis on conservation efforts and methods

Competitive Horseback Riding, USEF/USHJA National Competitions 2006-present

- Handled all the care of personal horses at home and at competitions
- Competed personal horses in nationally ranked competitions across the country
- Won numerous awards and championships including national year-end rankings

PROFESSIONAL EXPERIENCE

Graduate Assistant, SHSU, Huntsville, TX 2015-2016

- Administrative Assistant for SHSU Department of Agricultural Sciences & Engineering Technology
- Proctored exams, made interdepartmental deliveries, assisted both staff and faculty with a variety of jobs and duties for the department

Phi Kappa Phi, TSU, Kirksville, MO 2013-2016

- Member of the nation's largest, oldest, and most selective honor society for all academic disciplines

Sigma Alpha, TSU, Kirksville, MO 2012-2015

- Cooperated with members in yearly fundraiser to raise an average of $4,000
- Registered for and organized intramural sports teams for the sorority
- Attended professional meetings and events

PROFESSIONAL EXPERIENCE (continued)

Pre-Veterinary Club, TSU, Kirksville, MO 2011-2014
Secretary: Fall 2012-Spring 2013 Treasurer: Fall 2013-Spring 2014

- Developed professional skills by attending and participating in bi-weekly meetings including listening to various lectures
- Taught classes for the Adair County 4H club

SCHOLARSHIPS AND AWARDS

Allen and Joan Triplett Scholarship 2016-2017
$1,000 awarded by the Department of Agricultural Sciences and Engineering Technology at SHSU.
SHSU College of Sciences Special Graduate Scholarship Award 2016-2017
$1,500 per semester for 2016 and Spring 2017
USHJA/IHSA Sportsmanship Award Winner, Zone 7, Region 5 2015
Teresa L. McDonald Horsemanship Challenge National Competitor 2015
TruScholar Recipient 2014
$3000 to conduct original research resulting in "Evaluation of inter-day variation of horses on total fecal collection"
Missouri Bright Flight Scholarship 2011-2015
Up to $3,000 annually based on GPA and ACT scores
AT&T Foundation Scholarship 2011-2013
$1,500 annually
USHJA Affiliate Youth Sportsmanship Award, Zone 7
2011

RELEVANT COURSEWORK

Animal Physiology
Reproductive Physiology
Organic Chemistry
Biochemistry: Metabolism
Equine Reproductive Practicum
Statistics
Genetics
Animal Health
Animal Nutrition
Microbiology

SKILLS & TECHNIQUES

Equine Ultrasounding	Total Fecal Collection	Artificial Insemination
IV/IM Injections	Equine/Bovine Blood Collection	Western Blot Testing
Statistical Analysis System	YSI Glucose/Lactate Analysis	Semen Evaluation

GRADUATE RECORD EXAMINATION

Verbal 160 (85%) Quantitative 158 (70%) Analytical Writing 5.0 (93%)